# Supplementary Materials

# H2A mono-ubiquitination differentiates FACT's functions in nucleosome assembly and disassembly

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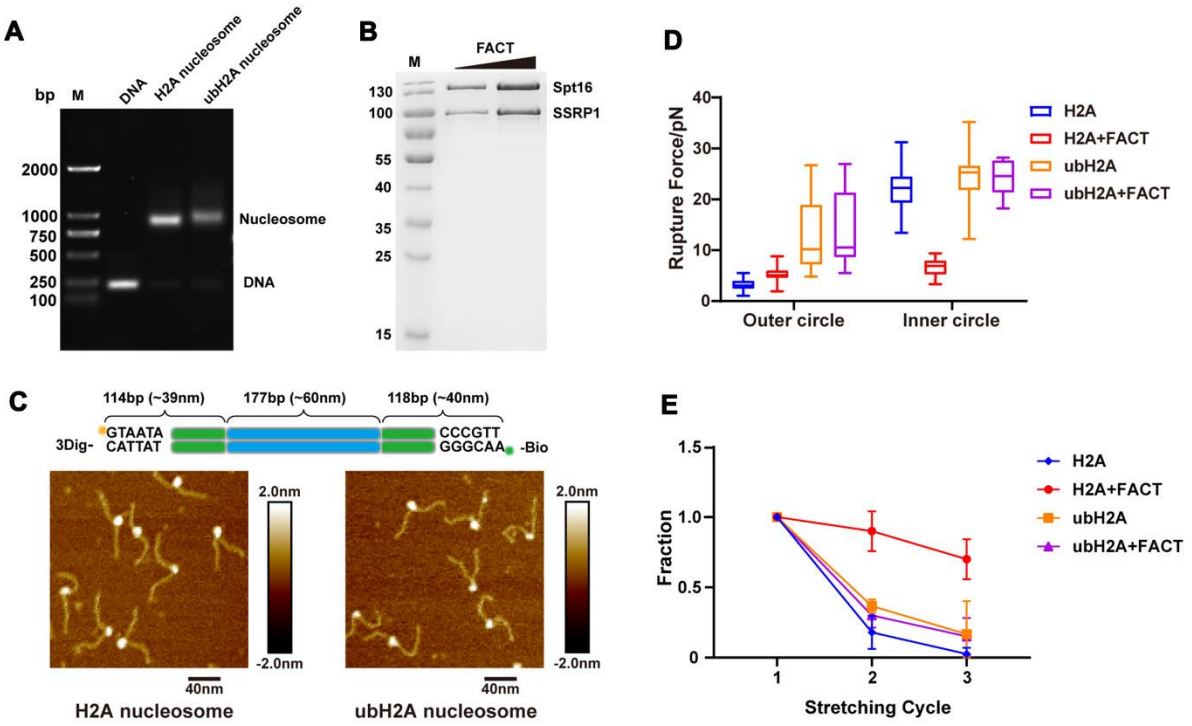


Figure S1. Related to Figure 1. (A) Gel electrophoresis mobility shift assay showed that H2A and ubH2A nucleosome were successfully reconstituted *in vitro*. (B) SDS-PAGE of purified FACT complex. (C) The constructs of DNA template used and atomic force microscopy (AFM) images of the reconstituted H2A and ub2HA nucleosomes for single molecule stretching experiment. (D) The statistic measurements of rupture forces of the outer and inner DNA wrap for H2A and ubH2A nucleosome with and without FACT. (E) The fraction of intact nucleosome in each stretching cycle. Error bars indicate SEM.

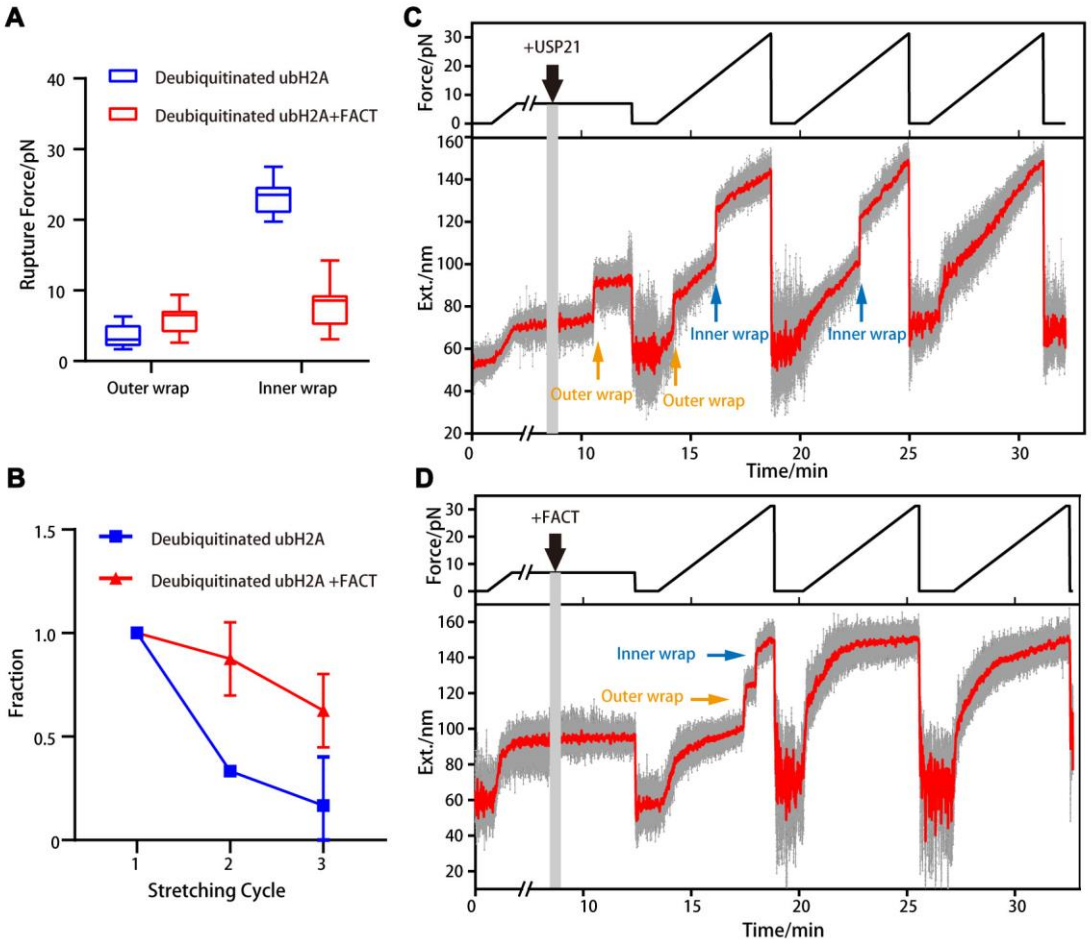


Figure S2. Related to Fig 2. (A) The statistic measurements of rupture forces for deubiquitinated ubH2A nucleosome with and without FACT. (B) The fraction of intact nucleosome in each stretching cycle. Error bars indicate SEM. (C and D) The real-time trace of deubiquitinating processes and repeated stretching process for ubH2A-nucleosome. The tension first increases to 7.0 pN continuously at 0.1 pN/s, and then holds at 7.0 pN for 3 min before the injection of USP21 (C) or FACT (D). After observing the deubiquitination process, the tension was released to 0 pN for 3 min, and then stretch to 32 pN at 0.1 pN/s for three times.

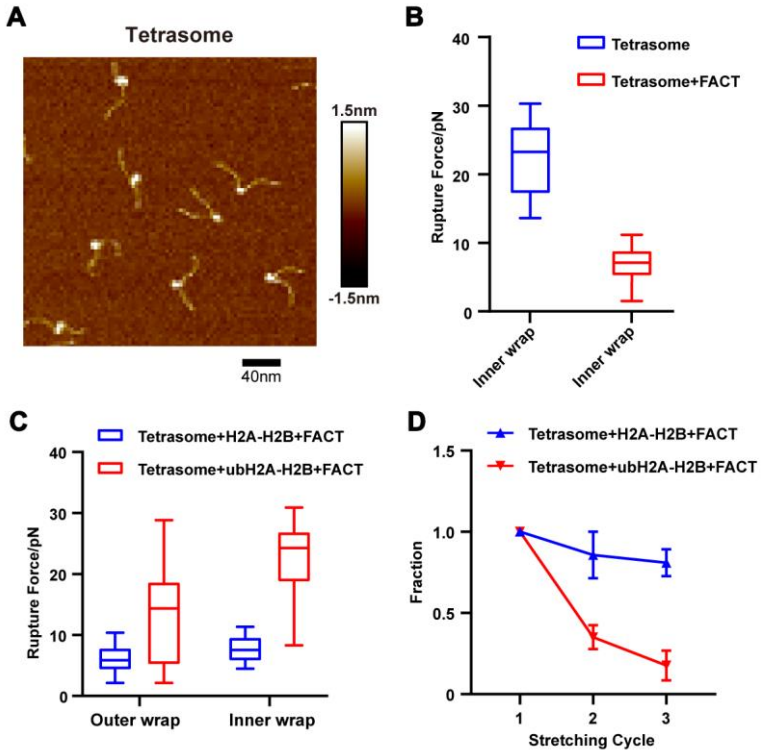


Figure S3. Related to Fig 3. (A) Atomic force image of mono-tetrasome reconstituted for single-molecule deposition experiment. (B) The statistic measurements of rupture forces for tetrasome with and without FACT. (C) The statistic measurements of rupture forces for the outer and inner DNA wrap for FACT-deposited H2A nucleosome and ubH2A nucleosome. (D) The fraction of intact nucleosome in each stretching cycle. Error bars indicate SEM.

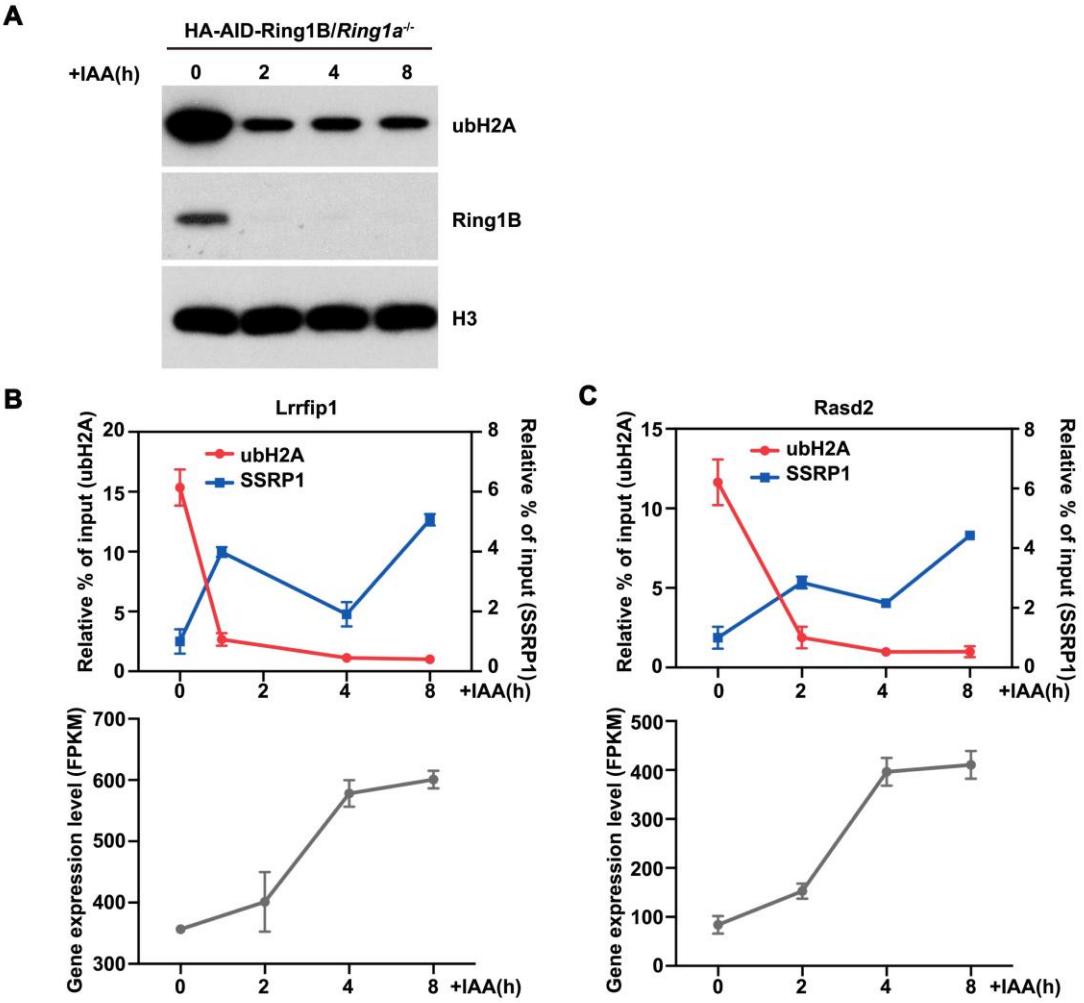


Figure S4. Related to Fig 4. (A) Representative immunoblot of ubH2A, Ring1B and H3 after a time course of IAA treatment in HA-AID-Ring1B mESCs. (B-C) ChIP-qPCR analysis of the level of ubH2A and SSRP1 on the promoter regions of Lrrfip1 and Rasd2 (up) and the related RNA-seq analysis of gene expression level with FPKM (fragments per kilobase of transcript per million fragments sequenced) (bottom) after IAA addition. The data represent means ± s.d. ChIP enrichments are normalized to the input. The relative % of input: ubH2A samples are normalized to the last time point, SSRP1 samples are normalized to the first time point.

Table S1. The statistics for the stretching experiments in the relative figure, with the number of stretching repeats and the fraction of nucleosome shown the behavior as indicated in the figure.

| **Figure number** | **Number of the stretching repeats** | **Fraction of behaviour as shown in the figure** |
|---|---|---|
| Fig 1D | 151 | 97.4% |
| Fig 1E | 83 | 82.3% |
| Fig 1F | 51 | 70% |
| Fig 1G | 35 | 87.5% |
| Fig 2B | 26 | 83.3% |
| Fig 2C | 32 | 62.5% |
| Fig 3C | 92 | 65.5% |
| Fig 3D | 37 | 90.0% |
| Fig 3E | 93 | 80.9% |
| Fig 3F | 155 | 82.3% |